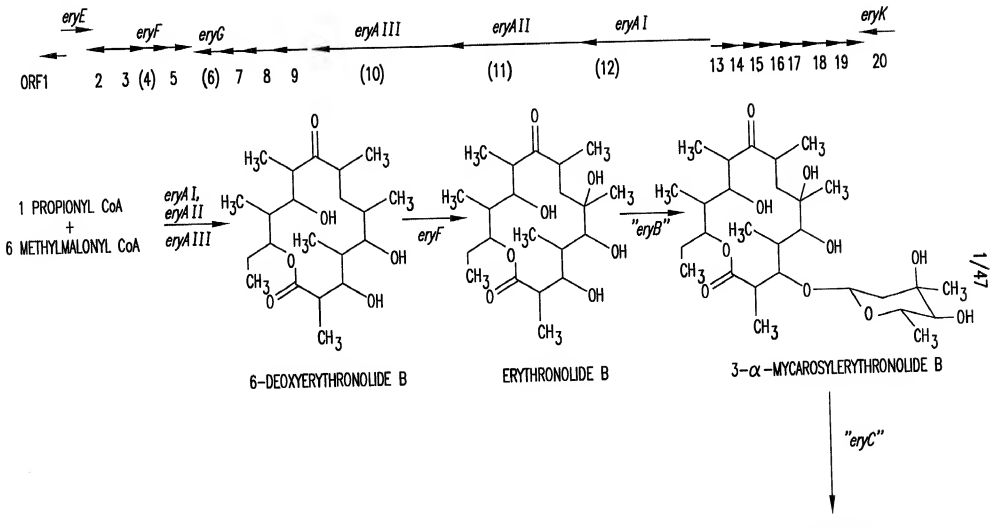


FIG.1a

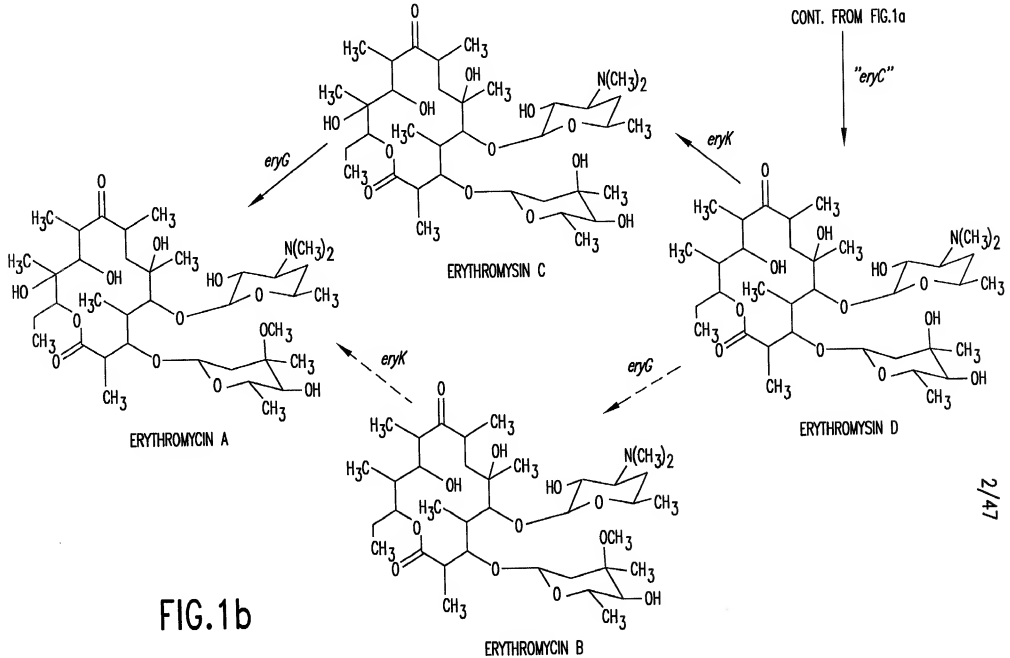

CONT. FROM FIG.1a
"eryC"
eryG
eryK
eryK
eryG
ERYTHROMYSIN C
ERYTHROMYSIN D
ERYTHROMYCIN A
ERYTHROMYCIN B

FIG.1b

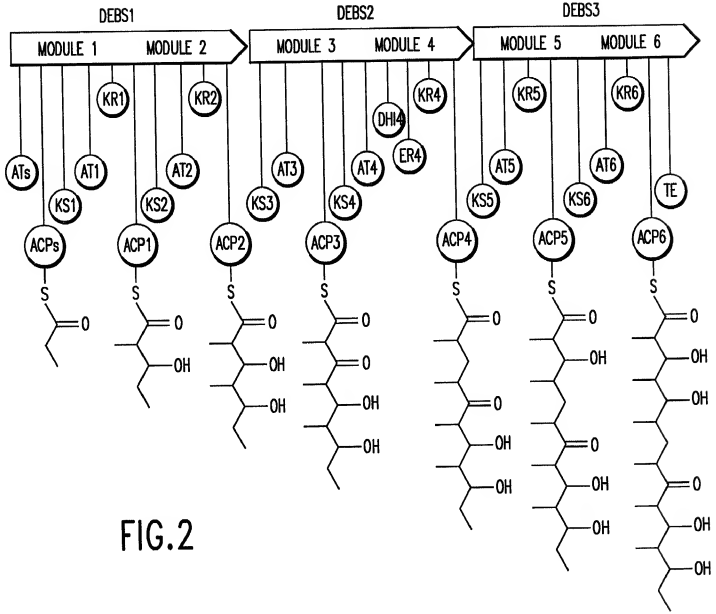

DEBS1
DEBS2
DEBS3
MODULE 1
MODULE 2
MODULE 3
MODULE 4
MODULE 5
MODULE 6
ATs
KS1
AT1
KR1
KS2
AT2
KR2
KS3
AT3
KS4
AT4
DHI4
ER4
KR4
KS5
AT5
KR5
KS6
AT6
KR6
TE
ACPs
ACP1
ACP2
ACP3
ACP4
ACP5
ACP6
S
O
OH

FIG.2

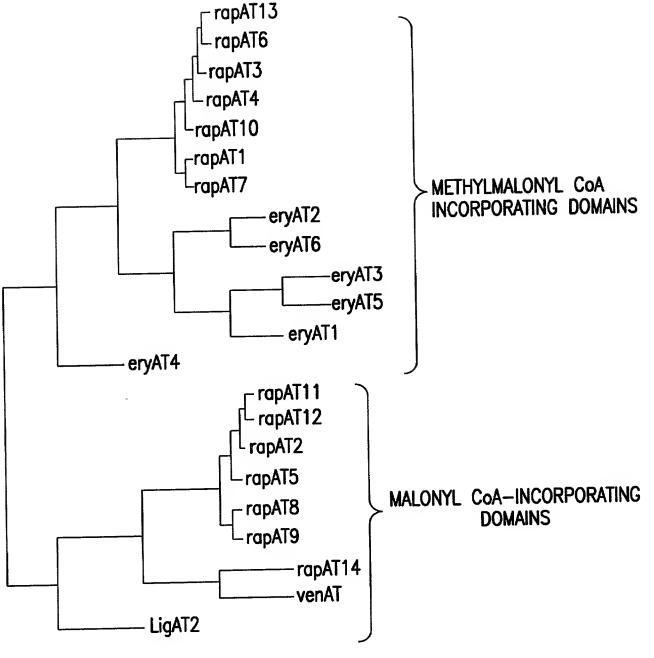
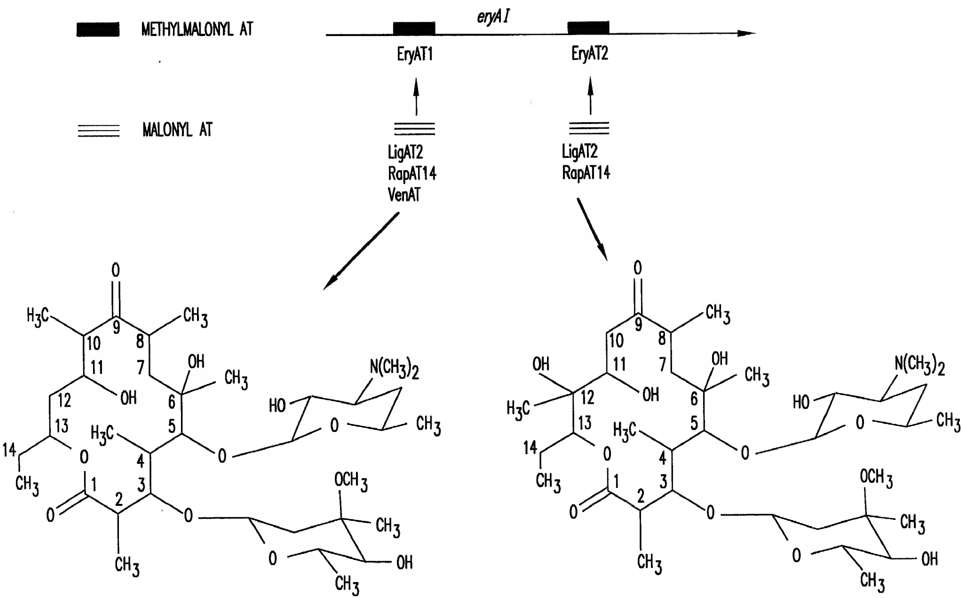

rapAT13
rapAT6
rapAT3
rapAT4
rapAT10
rapAT1
rapAT7
eryAT2
eryAT6
eryAT3
eryAT5
eryAT1
eryAT4
METHYLMALONYL CoA INCORPORATING DOMAINS
rapAT11
rapAT12
rapAT2
rapAT5
rapAT8
rapAT9
rapAT14
venAT
LigAT2
MALONYL CoA–INCORPORATING DOMAINS

FIG.3

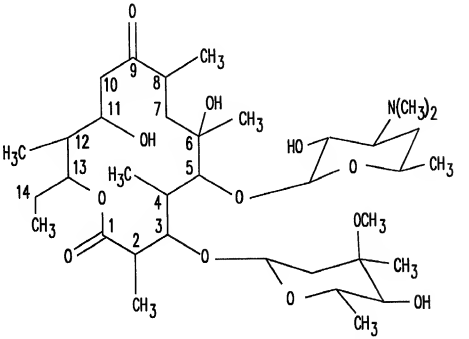


12-DESMETHYL, 12-DEOXYERYTHROMYCIN A

10-DESMETHYLERYTHROMYCIN A
AND
CONT. ON FIG.4b

FIG. 4a(1)

CONT. FROM FIG.4a



10-DESMETHYL-12-DEOXYERYTHROMYCIN A

FIG. 4a(2)

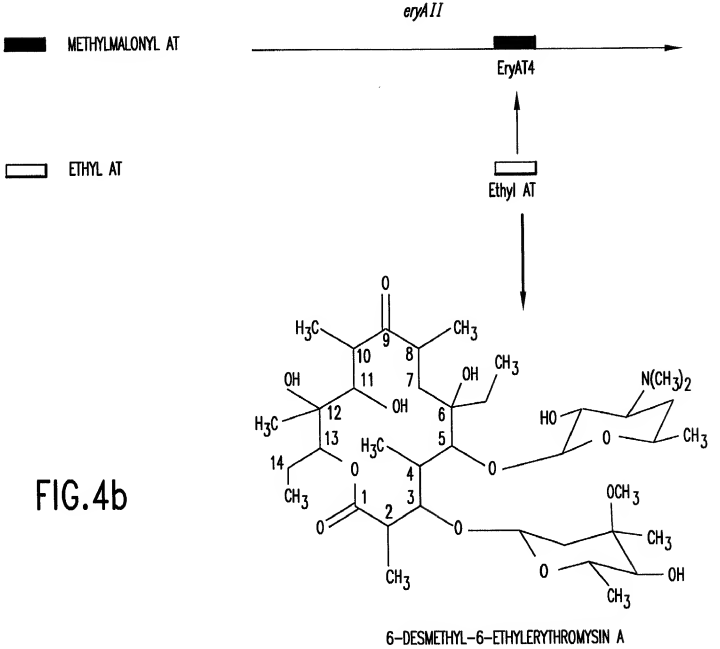


FIG.4b

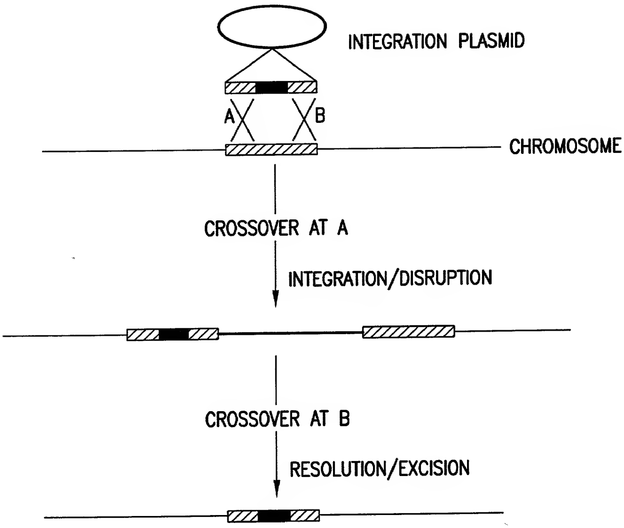

INTEGRATION PLASMID
A
B
CHROMOSOME
CROSSOVER AT A
INTEGRATION/DISRUPTION
CROSSOVER AT B
RESOLUTION/EXCISION

FIG.5

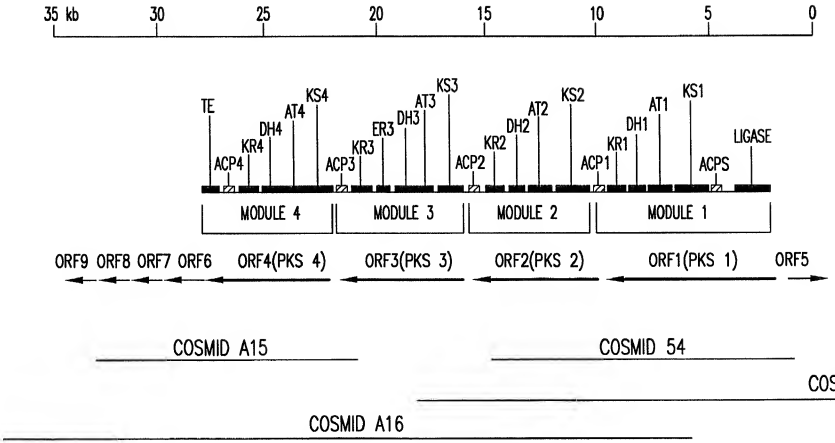
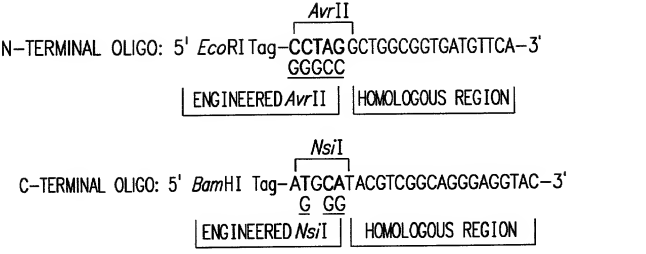


FIG.6

```
GGGCCGCTGGCGGTGATGTTCACCGGACAGGGCTCCCAACGCCCCGGCATGGGACGACAG  60
G  P  L  A  V  M  F  T  G  Q  G  S  Q  R  P  G  M  G  R  Q   20
TTGTACGAGCACTTCCCCGTCTSCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC  120
L  Y  E  H  F  P  V  F  A  Q  A  L  D  E  V  F  A  L  A  T   40
CCCGGACTACGCGAGGTGATGTTCGACCCCGACCAGGCCGAAACACTCCAACGCACCGAC  180
P  G  L  R  E  V  M  F  D  P  D  Q  A  E  T  L  Q  R  T  D   60
CACGCCCAGATCGCCCTGTTCGCCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG  240
H  A  Q  I  A  L  F  A  F  E  T  A  L  Y  R  L  W  E  S  W   80
GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTCGGAGAAATCACCGCAGCCCACGTC  300
G  L  R  P  D  M  V  C  G  H  S  V  G  E  I  T  A  A  H  V   100
TCCGGCACCCTCACCCTCCCCGACGCCGTCCACCTCGTCACCACACGCGGCACCCTCATG  360
S  G  T  L  T  L  P  D  A  V  H  L  V  T  T  R  G  T  L  M   120
CAAAACCTGCCCCCCGGCGGCGCCATGCTCGCCGTCGCCACCGACCCCCACACCCTCCAA  420
Q  N  L  P  P  G  G  A  M  L  A  V  A  T  D  P  H  T  L  Q   140
CCCCACCTCGACAACCACCACGACACCATCTCCATCGCCGCCATCAACGGCCCCCACGCC  480
P  H  L  D  N  H  H  D  T  I  S  I  A  A  I  N  G  P  H  A   160
ACCGTCCTCTCCGGCGACCGCACCACCCTCCACCACATCGCCACCCAACTCAACACCAAA  540
T  V  L  S  G  D  R  T  T  L  H  H  I  A  T  Q  L  N  T  K   180
ACCAACTGGCTCAACGTCAGCCACGCCTTCCACTCCCCCCTCATGCAACCCATCCTCCAA  600
T  N  W  L  N  V  S  H  A  F  H  S  P  L  M  Q  P  I  L  Q   200
CCCTTCACCACCACCCTCAACACCCTCACCCACCACCCCCCACACACACCCCTCATCAGC  660
P  F  T  T  T  L  N  T  L  T  H  H  P  P  H  T  P  L  I  S   220
ATGCTCACCGCCACACCCACCCACCCCGACACCACCCACTGGACCCAGCACATCACCGCA  720
M  L  T  A  T  P  T  H  P  D  T  T  H  W  T  Q  H  I  T  A   240
CCCGTCCGCTACACCGACACCCTCCACCACCTCCACCACCACGGCATCACCACCTACCTC  780
P  V  R  Y  T  D  T  L  H  H  L  H  H  H  G  I  T  T  Y  L   260
GAAATCGGCCCCGACACCACCCTCACCGCCCTCGCCCGCACCACCCTCCCCACCACCACC  840
E  I  G  P  D  T  T  L  T  A  L  A  R  T  T  L  P  T  T  T   280
CACCTCATCCCCACCACCCGCCGCAACCACAACGAAGTCCGCAGCACGAACGAGGCGTTG  900
H  L  I  P  T  T  R  R  N  H  N  E  V  R  S  T  N  E  A  L   300
GGCAGGGTGTTCAGCGTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG  960
G  R  V  F  S  V  G  H  S  V  D  W  R  A  L  T  P  T  G  R   320
CGTACCTCCCTGCCGACGTACCCCT                                     985
R  T  S  L  P  T  Y  P                                        328
```

FIG.7

PCR OLIGOS:



PCR CLONING:

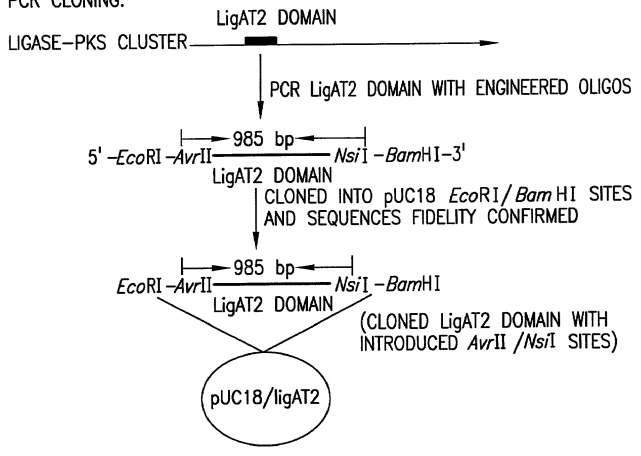


FIG.8

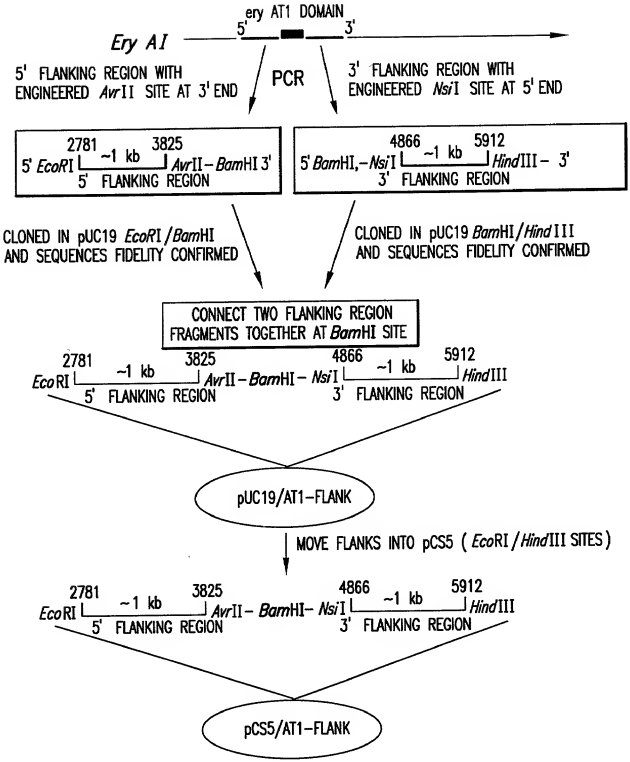

ery AT1 DOMAIN
5'
3'
Ery AI
5' FLANKING REGION WITH ENGINEERED AvrII SITE AT 3' END
PCR
3' FLANKING REGION WITH ENGINEERED NsiI SITE AT 5' END
2781
~1 kb
3825
5' EcoRI
AvrII – BamHI 3'
5' FLANKING REGION
4866
~1 kb
5912
5' BamHI, – NsiI
HindIII – 3'
3' FLANKING REGION
CLONED IN pUC19 EcoRI / BamHI AND SEQUENCES FIDELITY CONFIRMED
CLONED IN pUC19 BamHI / HindIII AND SEQUENCES FIDELITY CONFIRMED
CONNECT TWO FLANKING REGION FRAGMENTS TOGETHER AT BamHI SITE
2781
~1 kb
3825
4866
~1 kb
5912
EcoRI
AvrII – BamHI – NsiI
HindIII
5' FLANKING REGION
3' FLANKING REGION
pUC19/AT1–FLANK
MOVE FLANKS INTO pCS5 (EcoRI / HindIII SITES)
2781
~1 kb
3825
4866
~1 kb
5912
EcoRI
AvrII – BamHI – NsiI
HindIII
5' FLANKING REGION
3' FLANKING REGION
pCS5/AT1–FLANK

FIG.9

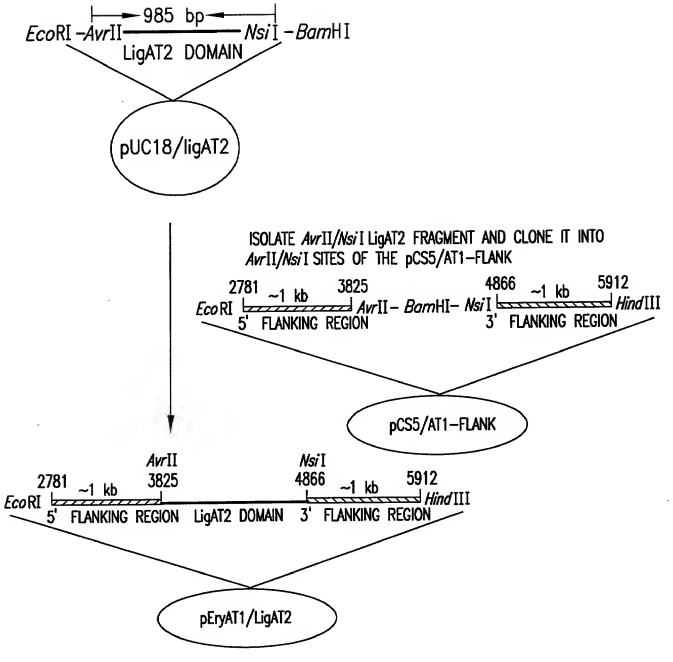

985 bp
EcoRI -AvrII- NsiI -BamHI
LigAT2 DOMAIN
pUC18/ligAT2
ISOLATE AvrII/NsiI LigAT2 FRAGMENT AND CLONE IT INTO AvrII/NsiI SITES OF THE pCS5/AT1-FLANK
2781 ~1 kb 3825
EcoRI AvrII- BamHI- NsiI
5' FLANKING REGION
4866 ~1 kb 5912
HindIII
3' FLANKING REGION
pCS5/AT1-FLANK
AvrII
NsiI
2781 ~1 kb 3825
4866 ~1 kb 5912
EcoRI
HindIII
5' FLANKING REGION LigAT2 DOMAIN 3' FLANKING REGION
pEryAT1/LigAT2

FIG.10

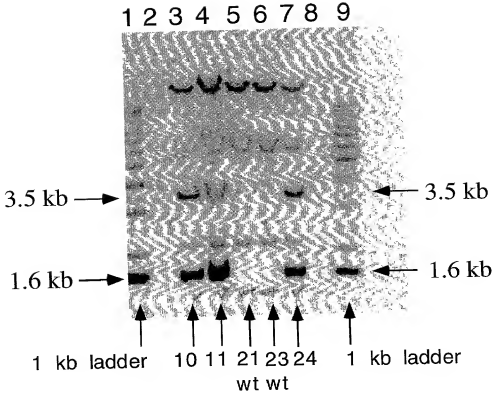

1 2 3 4 5 6 7 8 9
3.5 kb
1.6 kb
3.5 kb
1.6 kb
1 kb ladder
10 11 21 23 24
wt wt
1 kb ladder

FIG. 11

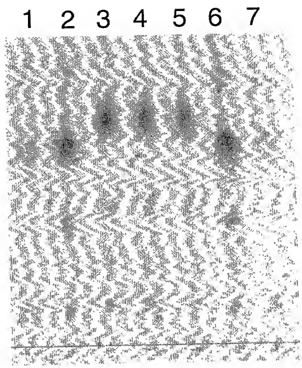


FIG. 12

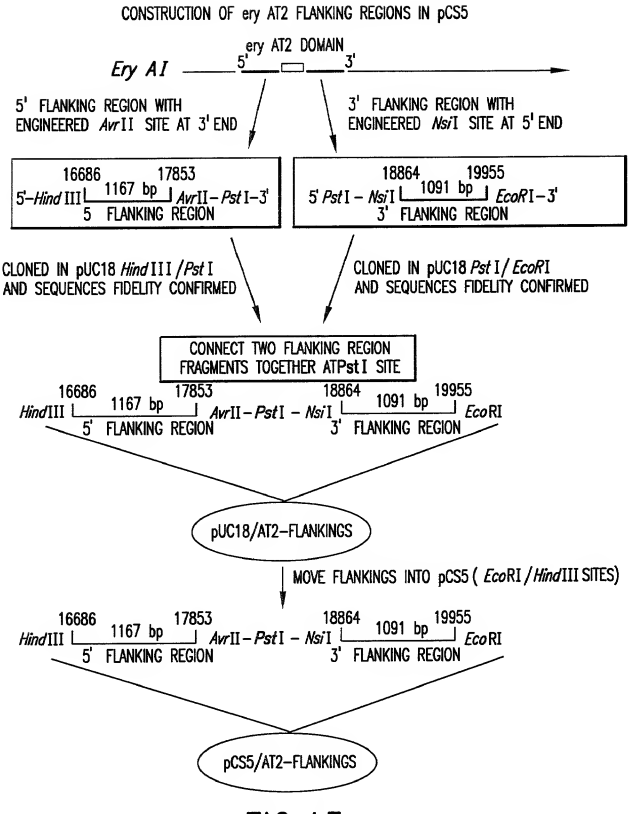

CONSTRUCTION OF ery AT2 FLANKING REGIONS IN pCS5
ery AT2 DOMAIN
Ery AI
5'
3'
5' FLANKING REGION WITH ENGINEERED AvrII SITE AT 3' END
3' FLANKING REGION WITH ENGINEERED NsiI SITE AT 5' END
16686
17853
5'-HindIII
1167 bp
AvrII-PstI-3'
5' FLANKING REGION
18864
19955
5' PstI - NsiI
1091 bp
EcoRI-3'
3' FLANKING REGION
CLONED IN pUC18 HindIII/PstI AND SEQUENCES FIDELITY CONFIRMED
CLONED IN pUC18 PstI/EcoRI AND SEQUENCES FIDELITY CONFIRMED
CONNECT TWO FLANKING REGION FRAGMENTS TOGETHER ATPstI SITE
HindIII
AvrII-PstI - NsiI
EcoRI
pUC18/AT2-FLANKINGS
MOVE FLANKINGS INTO pCS5 (EcoRI/HindIII SITES)
pCS5/AT2-FLANKINGS

FIG.13

SCHEME FOR CONSTRUCTION OF pEryAT2/LigAT2 INTEGRATION PLASMID

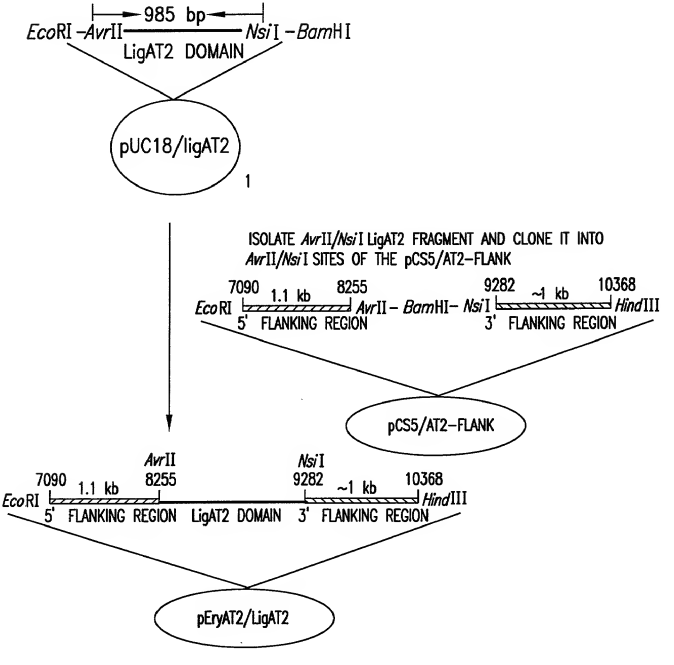


FIG.14

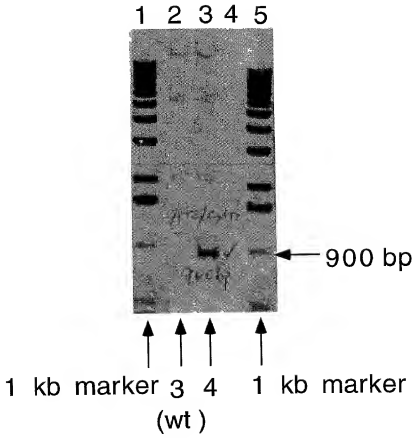


FIG. 15

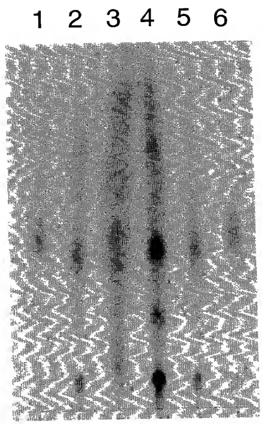


FIG.16

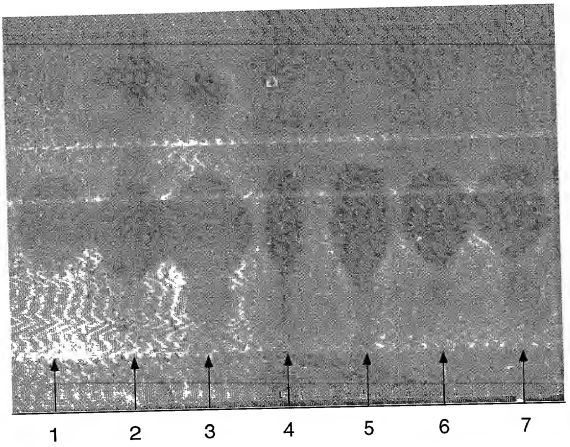


FIG. 17

```
CCTAGGACGGCAGTCCTGCTCACCGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCGCGAA  60
P  R  T  A  V  L  L  T  G  Q  G  S  Q  R  Q  G  M  G  R  E   20

CTGTACGACCGGTCACCGGTGTTCGCCGCCTCGTTCGACGCGATCTGCGCTCAACTCGAC  120
L  Y  D  R  S  P  V  F  A  A  S  F  D  A  I  C  A  Q  L  D   40

GGGCAACTGCCTCGTCCCCTCAAGGACGTTCTCTTCGCCCCCGAGGGGTCGGAGGACGCC  180
G  Q  L  P  R  P  L  K  D  V  L  F  A  P  E  G  S  E  D  A   60

GCGCTCASCGACCGTACGGTGTTCACACAGGCGGCTCTGTTCGCCGTGGAGACCTCCCTG  240
A  L  I  D  R  T  V  F  T  Q  A  A  L  F  A  V  E  T  S  L   80

TTCCGGCTGTTCGAGGCCCACGGCCTCGSCCCCGACTACCTCASCGGCCACTCCATCGGC  300
F  R  L  F  E  A  H  G  L  V  P  D  Y  L  I  G  H  S  I  G   100

GAAGTGACCGCGGCCCGCCTGGCCGGGGTCCTCGATCTGGCCGACGCGTGCGTCCTGGTC  360
E  V  T  A  A  H  L  A  G  V  L  D  L  A  D  A  C  V  L  V   120

GCCCACCGCGGCCGCCTGATGCAGTCGGCCCGGGCCGGCGGCGCGATGGCCGCGGTCCAG  420
A  H  R  G  R  L  M  Q  S  A  R  A  G  G  A  M  A  A  V  Q   140

GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCCGTTCCCGTGGCC  480
A  S  E  D  E  V  R  E  A  L  A  T  F  D  D  A  V  A  V  A   160

GGAGTCAACGGCCCGAACGCCACCGTCGTCTCCGGCGACGAGGACGCGGTCGAGCGGCTG  540
G  V  N  G  P  N  A  T  V  V  S  G  D  E  D  A  V  E  R  L   180

GTCGCGCGCTGGCGCGAGCAGGGCAGGCGGACGAAGCGGCTGCCGGTCAGCCACGCCTTC  600
V  A  R  W  R  E  Q  G  R  R  T  K  R  L  P  V  S  H  A  F   200

CACTCGCCGCACATGGACGGGATCGTCGACGAGTTCGTCACCGCCGTCTCCGGGCTCACC  660
H  S  P  H  M  I  G  I  V  D  E  F  V  T  A  V  S  G  L  T   220

TTCCGCTCCCCGACGLTCCCGGTCGTCTCCAACGTCACCGGGACCCTCGCCACCGTCGAC  720
F  R  S  P  T  I  P  V  V  S  N  V  T  G  T  L  A  T  V  D   240

CACCTGACCTCGCCCGCGTACTGGGCACGCCACATCCGCGAGGCCGTGCGCTTCGCCGAC  780
Q  L  T  S  P  A  Y  W  A  R  H  I  R  E  A  V  R  F  A  D   260

GGGGTGCGGTACCTGGAGGGCGAGGGCGTCACCGAATGGCTGGAGCTCGGGCCCGACGGC  840
G  V  R  Y  L  E  G  E  G  V  T  E  W  L  E  L  G  P  D  G   230

GTTCTCGTCGCCCTGGTCGAGGACTGCCTGGCGAAGGAGGCCGGCATCGCTCGCGTCCGCC  900
V  L  V  A  L  V  E  D  C  L  A  K  E  A  G  S  L  A  S  A   300

CTGCGCAAGGGGGCGAGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCCGCGGTGCTG  960
L  R  K  G  A  S  E  P  H  T  V  G  A  A  M  A  R  A  V  L   320

CGCGGATCCGGCCCCGACTGGGCGGCGGTGTTCCCCGGCGCACGGCGGGTCGACCTTCCG  1020
R  G  S  G  P  D  W  A  A  V  F  P  G  A  R  R  V  D  L  P   340

ACGTATGCAT                                                    1030
T  Y  A                                                        343
```

FIG. 18

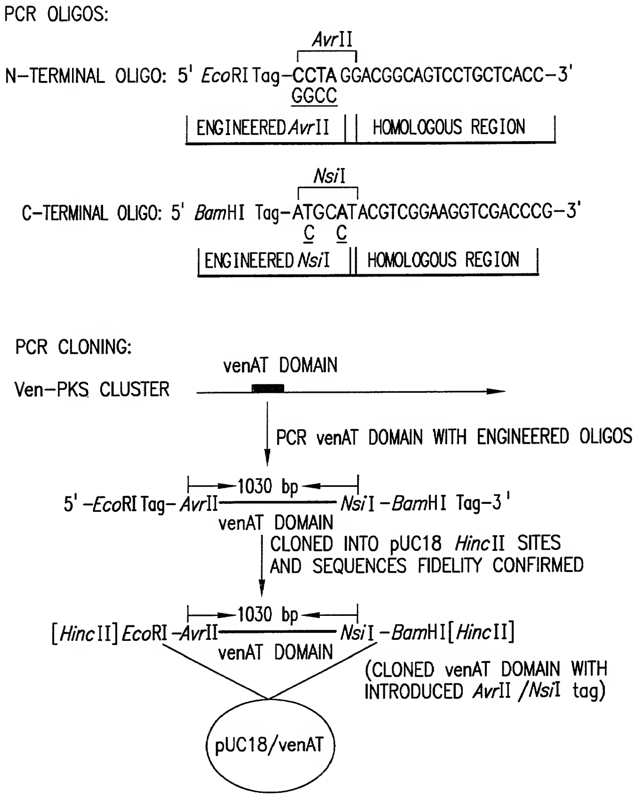

PCR OLIGOS:
AvrII
N-TERMINAL OLIGO: 5' EcoRI Tag–CCTA GGACGGCAGTCCTGCTCACC–3'
GGCC
ENGINEERED AvrII
HOMOLOGOUS REGION
NsiI
C-TERMINAL OLIGO: 5' BamHI Tag–ATGCAT ACGTCGGAAGGTCGACCCG–3'
C C
ENGINEERED NsiI
HOMOLOGOUS REGION
PCR CLONING:
venAT DOMAIN
Ven–PKS CLUSTER
PCR venAT DOMAIN WITH ENGINEERED OLIGOS
1030 bp
5'–EcoRI Tag–AvrII
NsiI–BamHI Tag–3'
venAT DOMAIN
CLONED INTO pUC18 HincII SITES
AND SEQUENCES FIDELITY CONFIRMED
1030 bp
[HincII] EcoRI–AvrII
NsiI–BamHI[HincII]
venAT DOMAIN
(CLONED venAT DOMAIN WITH
INTRODUCED AvrII /NsiI tag)
pUC18/venAT

FIG.19

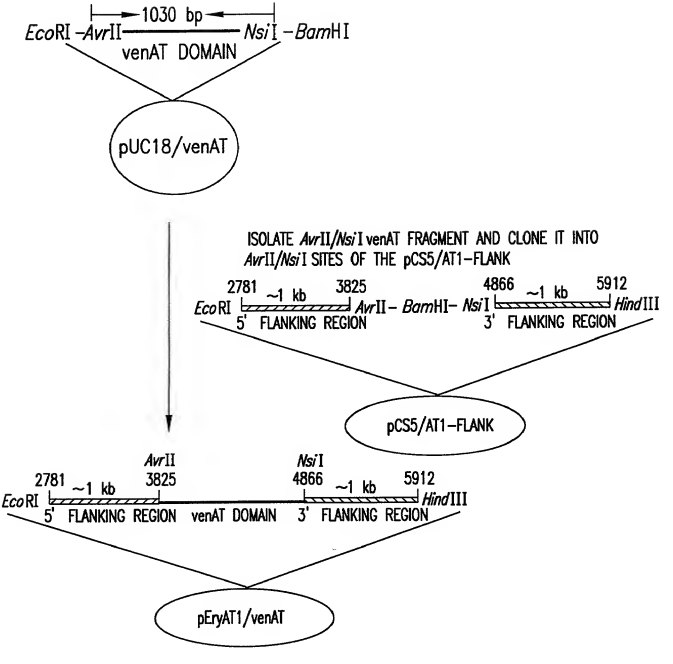

1030 bp
EcoRI -AvrII- NsiI -BamHI
venAT DOMAIN
pUC18/venAT
ISOLATE AvrII/NsiI venAT FRAGMENT AND CLONE IT INTO
AvrII/NsiI SITES OF THE pCS5/AT1-FLANK
2781 ~1 kb 3825
EcoRI AvrII- BamHI- NsiI
4866 ~1 kb 5912
HindIII
5' FLANKING REGION
3' FLANKING REGION
pCS5/AT1-FLANK
AvrII
NsiI
2781 ~1 kb 3825
4866 ~1 kb 5912
EcoRI
HindIII
5' FLANKING REGION venAT DOMAIN 3' FLANKING REGION
pEryAT1/venAT

FIG.20

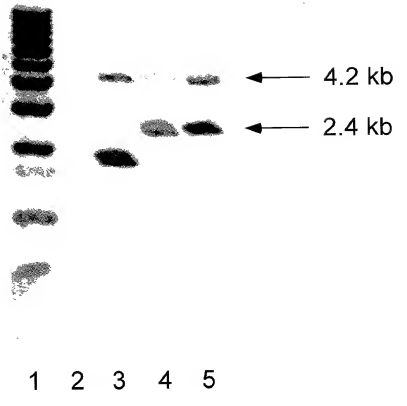


FIG. 21

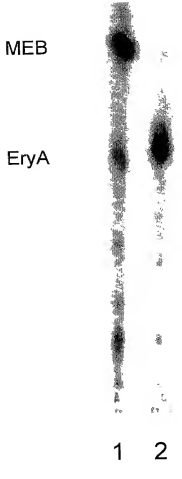
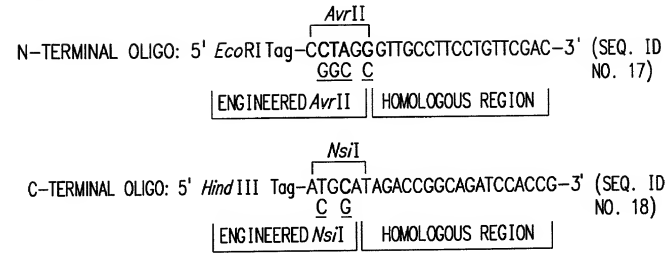


FIG. 22

PCR OLIGOS:



PCR CLONING:

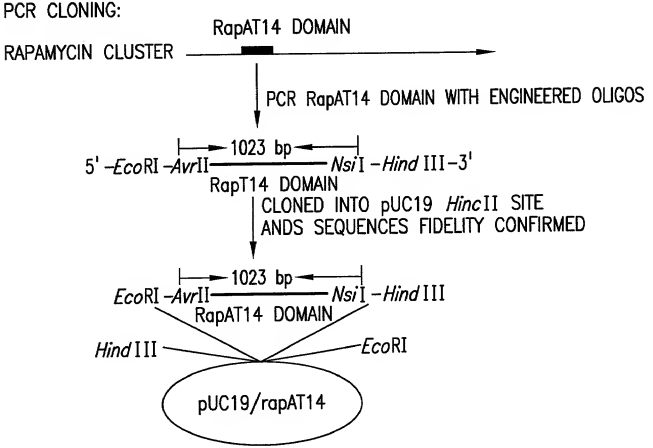


FIG.23

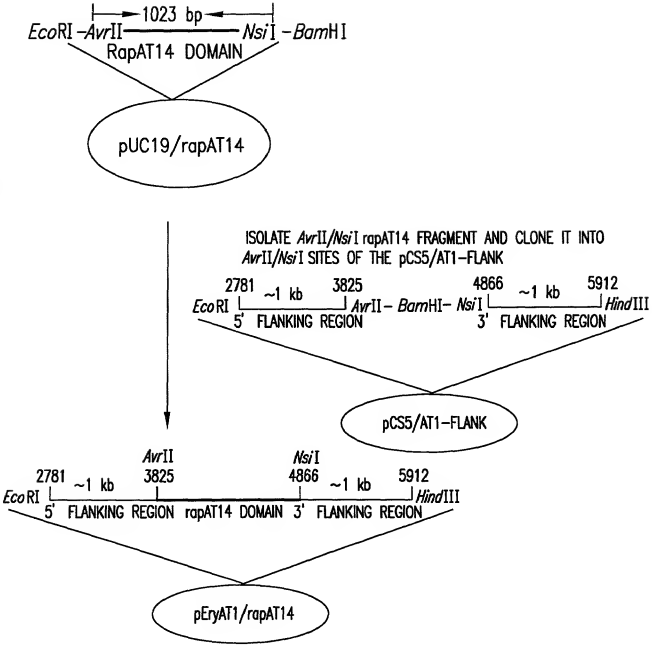

1023 bp
EcoRI -AvrII- NsiI -BamHI
RapAT14 DOMAIN
pUC19/rapAT14
ISOLATE AvrII/NsiI rapAT14 FRAGMENT AND CLONE IT INTO
AvrII/NsiI SITES OF THE pCS5/AT1-FLANK
2781 ~1 kb 3825
EcoRI
AvrII- BamHI- NsiI
4866 ~1 kb 5912
HindIII
5' FLANKING REGION
3' FLANKING REGION
pCS5/AT1-FLANK
AvrII
NsiI
2781 ~1 kb 3825 4866 ~1 kb 5912
EcoRI
HindIII
5' FLANKING REGION rapAT14 DOMAIN 3' FLANKING REGION
pEryAT1/rapAT14

FIG.24

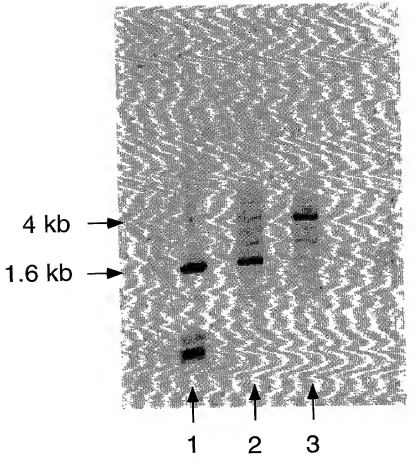


FIG. 25

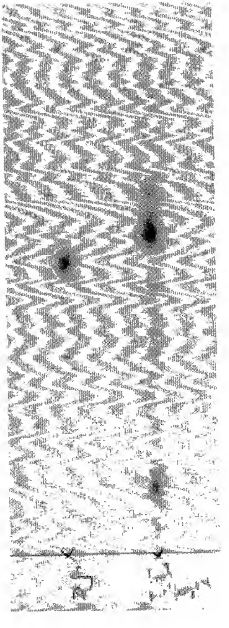


FIG. 26

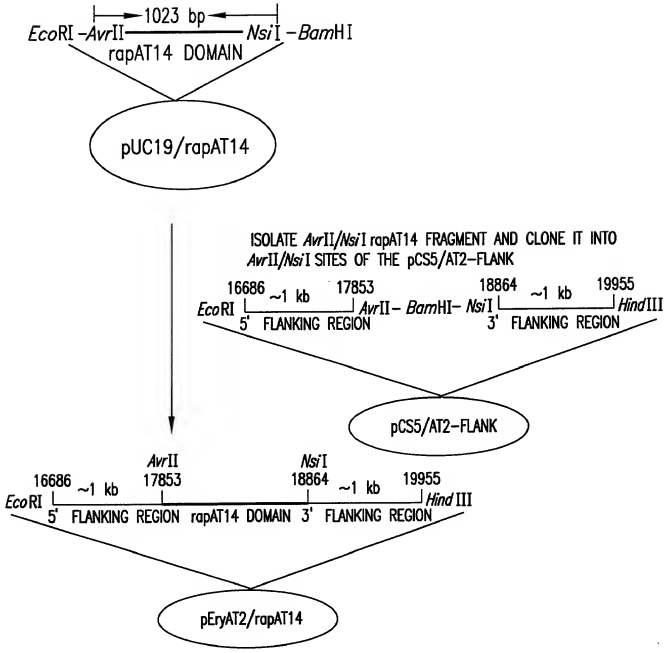

1023 bp
EcoRI -AvrII- NsiI -BamHI
rapAT14 DOMAIN
pUC19/rapAT14
ISOLATE AvrII/NsiI rapAT14 FRAGMENT AND CLONE IT INTO
AvrII/NsiI SITES OF THE pCS5/AT2-FLANK
16686 ~1 kb 17853
EcoRI 5' FLANKING REGION
AvrII- BamHI- NsiI
18864 ~1 kb 19955
HindIII
3' FLANKING REGION
pCS5/AT2-FLANK
AvrII
NsiI
16686 ~1 kb 17853 18864 ~1 kb 19955
EcoRI
HindIII
5' FLANKING REGION rapAT14 DOMAIN 3' FLANKING REGION
pEryAT2/rapAT14

FIG.27

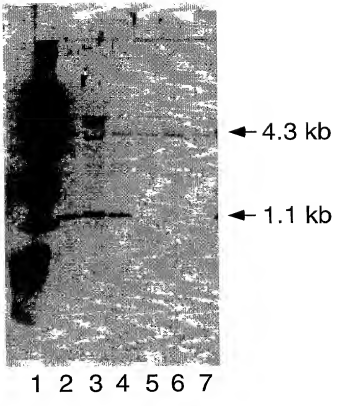


FIG. 28

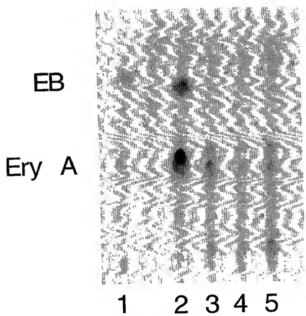


FIG. 29

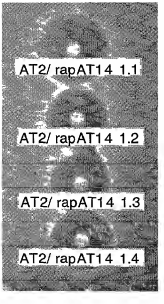


FIG. 30

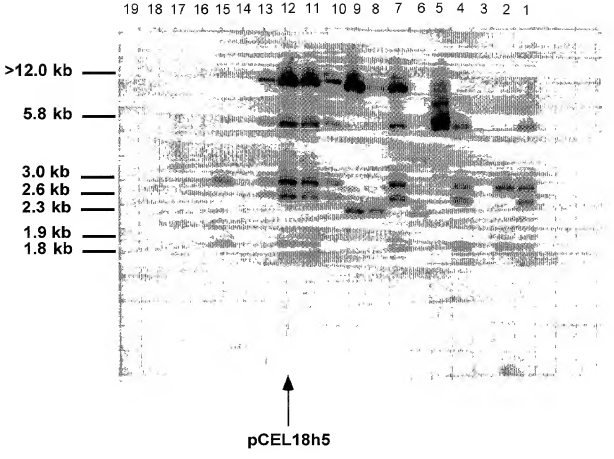

19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
>12.0 kb
5.8 kb
3.0 kb
2.6 kb
2.3 kb
1.9 kb
1.8 kb
pCEL18h5

FIG. 31

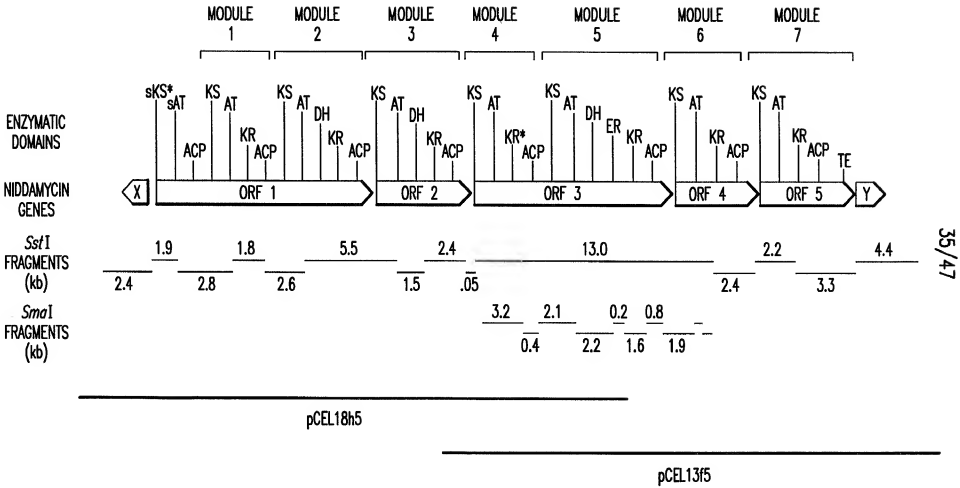

MODULE 1
MODULE 2
MODULE 3
MODULE 4
MODULE 5
MODULE 6
MODULE 7
ENZYMATIC DOMAINS
NIDDAMYCIN GENES
X
ORF 1
ORF 2
ORF 3
ORF 4
ORF 5
Y
SstI FRAGMENTS (kb)
1.9
1.8
5.5
2.4
13.0
2.2
4.4
2.4
2.8
2.6
1.5
.05
2.4
3.3
SmaI FRAGMENTS (kb)
3.2
2.1
0.2
0.8
0.4
2.2
1.6
1.9
pCEL18h5
pCEL13f5

FIG.32

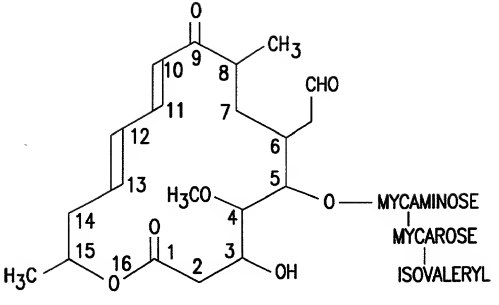


FIG.33

```
GCCGACCGTGTCGTGTTCGTGTTCCCCGGCCAGGGCTCGCAGTGGGCCGGAATGGCCGAG  60
A  D  R  V  V  F  V  F  P  G  Q  G  S  Q  W  A  G  M  A  E   20
GGGCTGCTGGAGCGGTCCGGCGCGTTCCGGAGTGCGGCCGACTCGTGCGACGCCGCGCTG 120
G  L  L  E  R  S  G  A  F  R  S  A  A  D  S  C  D  A  A  L  40
CGGCCGTACCTCGGCTGCTCGGTGCTGAGCGTGCTGCGCGGGGAACCGGACGCGCCCTCG 180
R  P  Y  L  G  W  S  V  L  S  V  L  R  G  E  P  D  A  P  S  60
CTCGACCGGGTCGACGTCGTGCAGCCGGTGCTGTTCACGATGATGGTCTCGCTCGCGGCG 240
L  D  R  V  D  V  V  Q  P  V  L  F  T  M  M  V  S  L  A  A  80
GTCTGGCGTGCGCTGGGCGTGGAACCGGCGGCGGTCGTCGGGCACTCGCAGGGTGAGATC 300
V  W  R  A  L  G  V  E  P  A  A  V  V  G  H  S  Q  G  E  I  100
GCCGCTGCCCATGTCGCCGGTGCGCTGTCGCTGGACGACTCGGCCCGGATCGTCGCCCTG 360
A  A  A  H  V  A  G  A  L  S  L  D  D  S  A  R  I  V  A  L  120
CGCAGTCGGGCGTGGCTCGGACTGGCGGGCAAGGGCGGCATGGTGGCGGTGCCGATGCCG 420
R  S  R  A  W  L  G  L  A  G  K  G  G  M  V  A  V  P  M  P  140
GCGGAGGAGCTGCGGCCGCGGCTGGTGACGTGGGGGGACCGTCTGGCCGTCGCCGCCGTC 480
A  E  E  L  R  P  R  L  V  T  W  G  D  R  L  A  V  A  A  V  160
AACAGCCCCGGTTCCTGCGCCGTCGCAGGCGACCCGGAGGCGCTGGCCGAACTGGTGGCG 540
N  S  P  G  S  C  A  V  A  G  D  P  E  A  L  A  E  L  V  A  180
CTGCTGACCGGTGAGGGGGTGCACGCCCGGCCGATCCCCGGCGTCGACACGGCGGGCCAC 600
L  L  T  G  E  G  V  H  A  R  P  I  P  G  V  D  T  A  G  H  200
TCGCCGCAGGTGGACGCGTTGCGGGCTCATCTGCTGGAGGTGCTGGCCCCGGTCGCCCCC 660
S  P  Q  V  D  A  L  R  A  H  L  L  E  V  L  A  P  V  A  P  220
CGACCGGCCGACATCCCGTTCTACTCGACGGTGACCGGCGGGCTGCTGGACGGCACCGAG 720
R  P  A  D  T  P  F  Y  S  T  V  T  G  G  L  L  D  G  T  E  240
CTGGACGCGACGTACTGGTACCGCAACATGCGCGAGCCCGTCGAGTTCGAGCGGGCCACA 780
L  D  A  T  Y  W  Y  R  N  M  R  E  P  V  E  F  E  R  A  T  260
CGGGCGCTGATCGCCGACGGGCACGACGTCTTCCTGGAGACGAGCCCGCATCCCATGCTG 840
R  A  L  I  A  D  G  H  D  V  F  L  E  T  S  P  H  P  M  L  280
GCCGTGGCGCTGGAGCAGACGGTCACCGACGCCGGCACCGACGCGGCGGTGCTCGGGACC 900
A  V  A  L  E  Q  T  V  T  D  A  G  T  D  A  A  V  L  G  T  300
CTGCGCCGCCGCCACGGCGGTCCTCGCGCGCTGGCCCTGGCCGTCTGCCGCGCCTTCGCG 960
L  R  R  R  H  G  G  P  R  A  L  A  L  A  V  C  R  A  F  A  320
CACGGCGTGGAGGTGGACCCCGAGGCGGTCTTCGGTCCGGGCGCACGGCCCGTGGAGTTG 1020
H  G  V  E  V  D  P  E  A  V  F  G  P  G  A  R  P  V  E  L  340
CCCACCTATCCG                                                 1032
P  T  Y  P                                                   344
```

FIG. 34

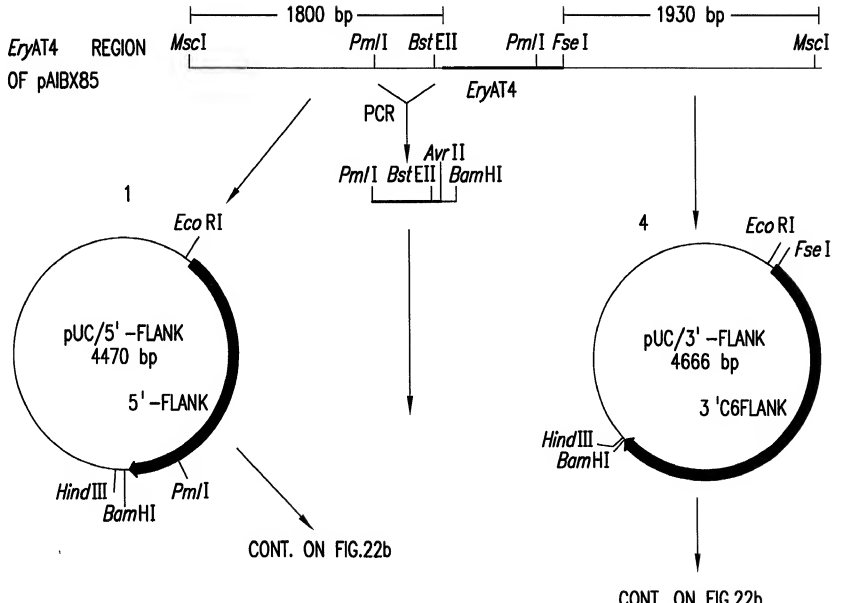

1800 bp
1930 bp
EryAT4 REGION OF pAIBX85
MscI
PmlI
BstEII
PmlI
FseI
MscI
EryAT4
PCR
AvrII
PmlI
BstEII
BamHI
1
EcoRI
pUC/5'-FLANK
4470 bp
5'-FLANK
HindIII
BamHI
PmlI
CONT. ON FIG.22b
4
EcoRI
FseI
pUC/3'-FLANK
4666 bp
3'C6FLANK
HindIII
BamHI
CONT. ON FIG.22b

FIG.35a

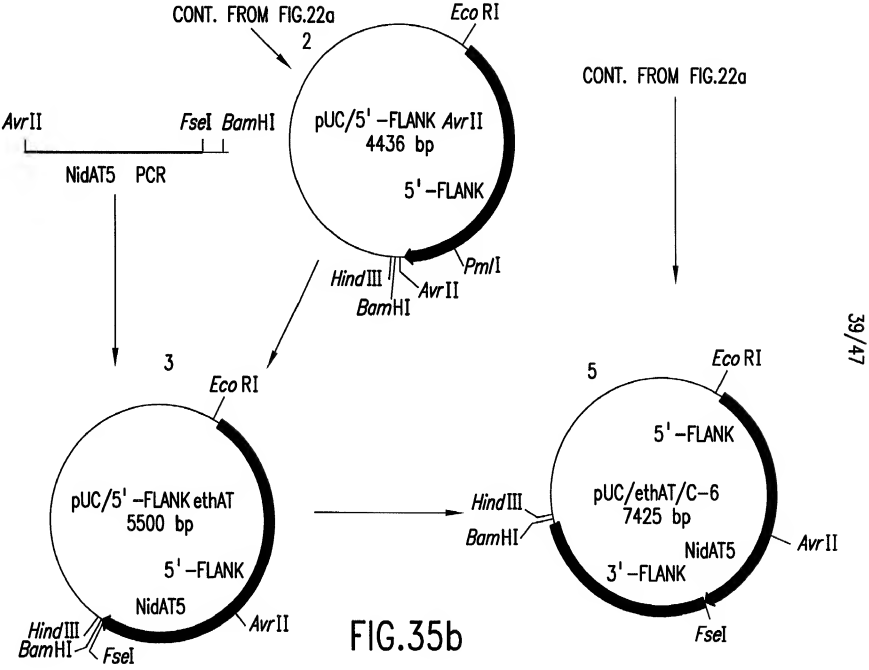
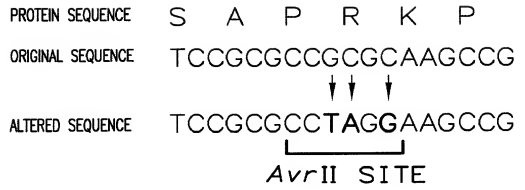
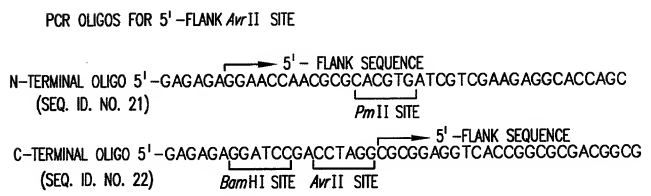

CONT. FROM FIG.22a
2
Eco RI
AvrII
FseI BamHI
pUC/5'-FLANK AvrII
4436 bp
NidAT5 PCR
5'-FLANK
PmlI
HindIII
AvrII
BamHI
CONT. FROM FIG.22a
3
Eco RI
pUC/5'-FLANK ethAT
5500 bp
5'-FLANK
NidAT5
AvrII
HindIII
BamHI
FseI
5
Eco RI
5'-FLANK
pUC/ethAT/C-6
7425 bp
HindIII
BamHI
NidAT5
AvrII
3'-FLANK
FseI

FIG.35b


PROTEIN SEQUENCE S A P R K P
ORIGINAL SEQUENCE TCCGCGCCGCGCAAGCCG
ALTERED SEQUENCE TCCGCGCCTAGGAAGCCG
AvrII SITE


PCR OLIGOS FOR 5'-FLANK AvrII SITE
5'- FLANK SEQUENCE
N-TERMINAL OLIGO 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCGAAGAGGCACCAGC
(SEQ. ID. NO. 21)
PmII SITE
5'-FLANK SEQUENCE
C-TERMINAL OLIGO 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG
(SEQ. ID. NO. 22)
BamHI SITE AvrII SITE

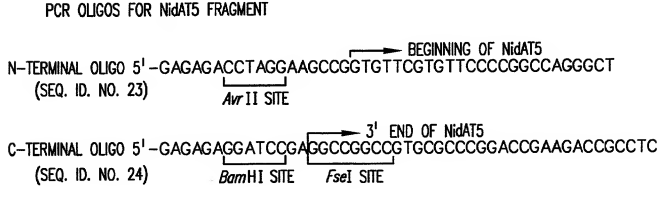

PCR OLIGOS FOR NidAT5 FRAGMENT
BEGINNING OF NidAT5
N-TERMINAL OLIGO 5'-GAGAGACCTAGGAAGCCGGTGTTCGTGTTCCCCGGCCAGGGCT
(SEQ. ID. NO. 23)
AvrII SITE
3' END OF NidAT5
C-TERMINAL OLIGO 5'-GAGAGAGGATCCGAGGCCGGCCGTGCGCCCGGACCGAAGACCGCCTC
(SEQ. ID. NO. 24)
BamHI SITE FseI SITE

FIG.36

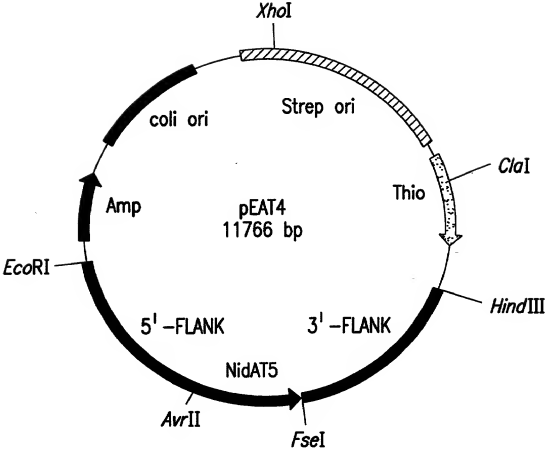


FIG.37

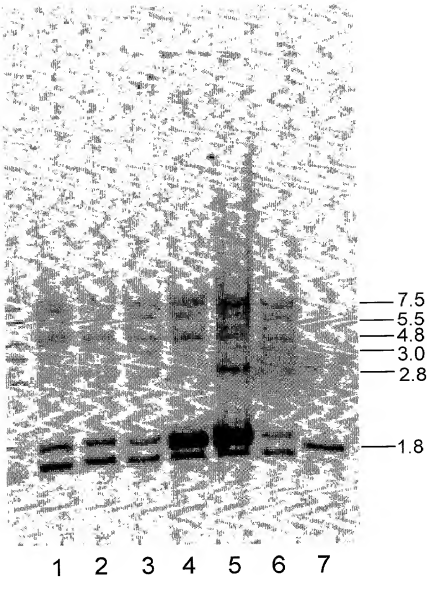


FIG. 38

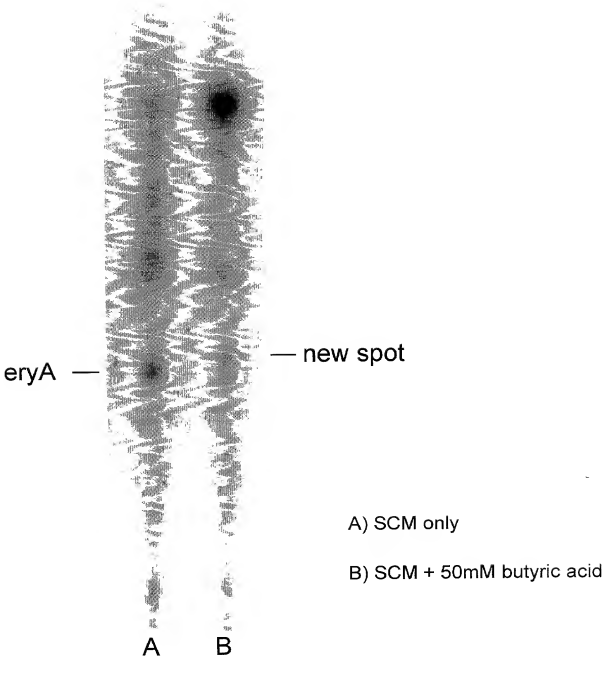


FIG. 39

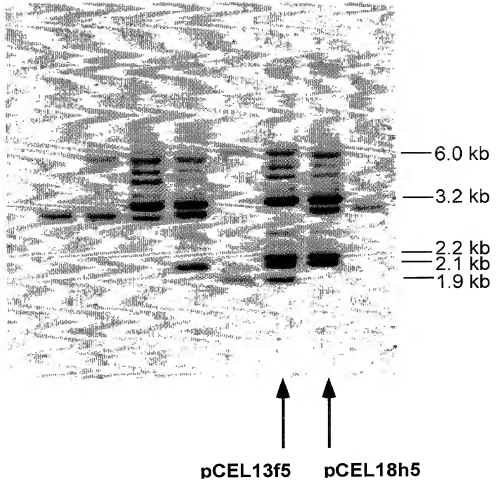


FIG. 40

```
CGCGCGCCTGCCTTCGTCTTTCCCGGGCAGGGCGCCCAGTGGGCCGGACTGGGAGCGCGG 60
R  A  P  A  F  V  F  P  G  Q  G  A  Q  W  A  G  L  G  A  R  20
CTCCTCGCGGACTCCCCCGTCTTCCGCCCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120
L  L  A  D  S  P  V  F  R  A  R  A  E  A  C  A  R  A  L  E  40
CCTCACCTCGACTGGTCGGTCCTCGACGTGCTGGCCGGCGCCCCGGGCACCCCTCCCATC 180
P  H  L  D  W  S  V  L  D  V  L  A  G  A  P  G  T  P  P  I  60
GACCGGGCCGACGTGGTGCAGCCGGTGCTGTTCACCACGATGGTCTCGCTGGCCGCCCTC 240
D  R  A  D  V  V  Q  P  V  L  F  T  T  M  V  S  L  A  A  L  80
TGGGAGGCCCACGGGGTGCGGCCGGCCGCGGTCGTGGGCCACTCCCAGGGCGAGGTGGCC 300
W  E  A  H  G  V  R  P  A  A  V  V  G  H  S  Q  G  E  V  A  100
GCGGCCTGCGTGGCCGGTGCCCTGTCGCTGGACGACGCTGCCCTGGTGATCGCCGGACGC 360
A  A  C  V  A  G  A  L  S  L  D  D  A  A  L  V  I  A  G  R  120
AGCAGGCTGTGGGGGCGGCTGGCCGGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420
S  R  L  W  G  R  L  A  G  N  G  G  M  L  A  V  M  A  P  A  140
GAGCGGATCCGTGAGCTGCTCGAACCATGGCGGCAGCGGATTTCGGTGGCGGCGGTCAAT 480
E  R  I  R  E  L  L  E  P  W  R  Q  R  I  S  V  A  A  V  N  160
GGCCCCGCCTCGGTCACCGTCTCCGGTGACGCGCTCGCGCTGGAGGAGTTCGGCGCGCGG 540
G  P  A  S  V  T  V  S  G  D  A  L  A  L  E  E  F  G  A  R  180
CTCTCCGCCGAGGGGGTGCTGCGCTGGCCGCTGCCGGGCGTCGACTTCGCCGGCCACTCG 600
L  S  A  E  G  V  L  R  W  P  L  P  G  V  D  F  A  G  H  S  200
CCGCAGGTGGAGGAGTTCC GC5CTGAGCTCCTGGACCTGCTCTCCGGCGTACGGCCGGC 660
P  Q  V  E  E  F  R  A  E  L  L  D  L  L  S  G  V  R  P  A  220
CCTTCGCGGATACCTTTCTTCTCCACCGTGACGGCGGGTCCTTGCGGCGGCGACCAGCTG 720
P  S  R  I  P  F  P  S  T  V  T  A  G  P  C  G  G  D  Q  L  240
GACGGGGCGTACTGGTACCGCAACACGCGCGAACCCGTGGAGTTCGACGCCACGGTCCGG 780
D  G  A  Y  W  Y  R  N  T  R  E  P  V  E  F  D  A  T  V  R  260
GCGCTGCTGCGTGCGGGCCATCACACGTTCATCGAGGTCGGTCCGCATCCGCTGCTCAAC 840
A  L  L  R  A  G  H  H  T  F  I  E  V  G  P  H  P  L  L  N  280
GCCGCGATCGACGAGATCGCAGCGGACGAGGGGGTAGCGGCCACGGCCCTGCATACGCTC 900
A  A  I  D  E  I  A  A  D  E  G  V  A  A  T  A  L  H  T  L  300
CAGCGGGGCGCTGGCGGCCTTGACCGCGTGCGCAACGCGGTGGGCGCCGCTTTCGCGCAC 960
Q  R  G  A  G  G  L  D  R  V  R  N  A  V  G  A  A  F  A  H  320
GGTGTCCGGGTCGACTGGAACGCCCTGTTCGAGGGCACCGGTGCGCGCAGGGTGCCGCTT 1020
G  V  R  V  D  W  N  A  L  F  E  G  T  G  A  R  R  V  P  L  340
CCCTCGTACGCCTTC                                              1035
P  S  Y  A  F                                                345
```

FIG. 41

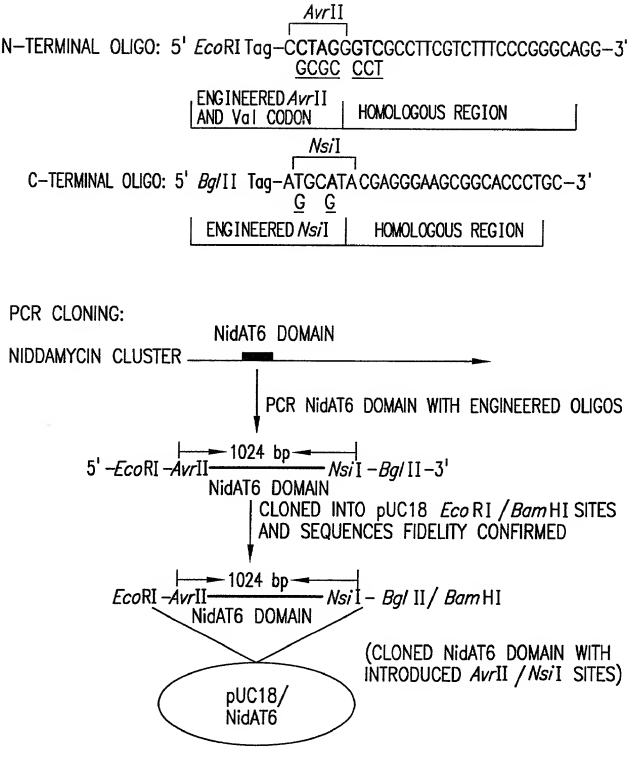

PCR OLIGOS:
AvrII
N–TERMINAL OLIGO: 5' EcoRI Tag–CCTAGGGTCGCCTTCGTCTTTCCCGGGCAGG–3'
GCGC CCT
ENGINEERED AvrII AND Val CODON
HOMOLOGOUS REGION
NsiI
C–TERMINAL OLIGO: 5' BglII Tag–ATGCATACGAGGGAAGCGGCACCCTGC–3'
G G
ENGINEERED NsiI
HOMOLOGOUS REGION
PCR CLONING:
NIDDAMYCIN CLUSTER
NidAT6 DOMAIN
PCR NidAT6 DOMAIN WITH ENGINEERED OLIGOS
1024 bp
5' –EcoRI –AvrII– NsiI –BglII–3'
NidAT6 DOMAIN
CLONED INTO pUC18 EcoRI / BamHI SITES
AND SEQUENCES FIDELITY CONFIRMED
1024 bp
EcoRI –AvrII– NsiI – BglII / BamHI
NidAT6 DOMAIN
(CLONED NidAT6 DOMAIN WITH
INTRODUCED AvrII / NsiI SITES)
pUC18/
NidAT6

FIG.42

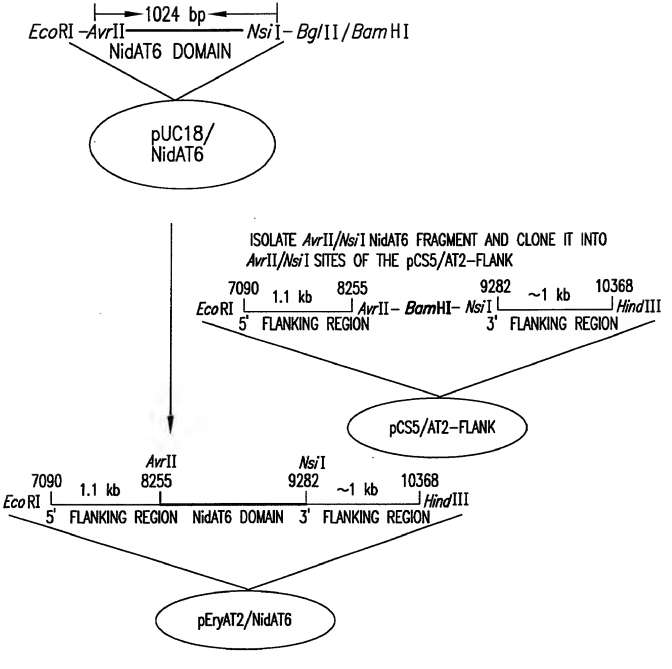

1024 bp
EcoRI -AvrII- NsiI- BglII/BamHI
NidAT6 DOMAIN
pUC18/
NidAT6
ISOLATE AvrII/NsiI NidAT6 FRAGMENT AND CLONE IT INTO
AvrII/NsiI SITES OF THE pCS5/AT2-FLANK
7090
1.1 kb
8255
9282
~1 kb
10368
EcoRI
AvrII- BamHI- NsiI
HindIII
5' FLANKING REGION
3' FLANKING REGION
pCS5/AT2-FLANK
AvrII
NsiI
7090
1.1 kb
8255
9282
~1 kb
10368
EcoRI
HindIII
5' FLANKING REGION
NidAT6 DOMAIN
3' FLANKING REGION
pEryAT2/NidAT6

FIG.43